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14. ABSTRACT

The work proposed for this award involved using the crystal structures of theextracellular domains of the EGF receptor (EGFR) and its homologsHER2/Neu/ErbB2, HER3/ErbB3, and HER4/ErbB4, which were recentlydetermined in my lab and elsewhere, to design a new class of inhibitors of thisfamily of receptors. These structures had shown that ligands bind to twoseparate surfaces in these receptors that are normally far apart in the absence ofligand. Binding ligand (e.g. EGF) requires a large conformational change in thereceptor to bring these surfaces close together, and it is this conformationalchange that then leads to receptor dimerization and initiation of a signalingcascade through activation of a cytoplasmic tyrosine kinase. Our idea had beento create through mutagenesis a ligand that bound more tightly to one of thebinding surfaces but not to the other. Theoretically, this ligand would bind to thereceptor but not induce the conformational change needed to activate thereceptor and thus serve an inhibitor of the receptor.

15. SUBJECT TERMS
ErbB Receptor Inhibitors

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I am responding to the email of February 22, 2007 from Dr. Katherine H. Moore USAMRMC indicating that the Progress Report for grant BC030254; W81XWH-04-1-0449 was deemed unacceptable. To my horror I have just discovered that **I incorporated the wrong year's progress report when composing the PDF version of the 2006 progress report**; I should have identified this mistake earlier. I used the 2005 report as a template for the 2006 report and left very similarly titled files in the same computer folder. Appended to these comments is the 2006 progress report prepared last fall. In reading the comments on the 2006 Progress Report, I also realize that I did not receive comments on the original 2005 report, which are referred to in the 2006 report. Thus, in addition to providing the original 2006 report prepared for last autumn, I will respond to comments in the 2005 report as well as provide an additional update on work since preparation of the actual 2006 report. I sincerely apologize for my mistake in uploading the wrong file.

Response to Specific Comments:

1. *"This second annual report is identical to the first annual report; no new work is reported."*

The second annual report was indeed identical to the first owing to an apparent mistake on my part when uploading the file last autumn. I sincerely regret this error. The actual 2006 progress report prepared last autumn is appended to this file and an update on results obtained since preparation of that file will also be added.

2. *"The purpose of the Key Research Accomplishments section is to report scientific findings. The PI misunderstood this and has reported mostly project milestones instead."*

This comment apparently refers to the 2005 report. I am not sure that I yet fully understand the distinction between "Key research Accomplishments" and "Project Milestones". The accomplishments listed are actual accomplishments not projections.

3. *"The original Statement of Work (SOW) called for the genetic engineering of specific mutants of ErbB1, ErbB2, ErbB3 and their protein ligands in order to: (1) produce a protein agonist for the receptor, and (2) explore structural features of receptor activation. Because the PI found that several other labs are pursuing these same objectives, he decided instead to solve the crystal structure of the HER2 cytoplasmic kinase domain and use the structure to design inhibitors. This comprises a profound change in the focus of the project, and following review of the first annual report, the Grants Manager requested the PI to submit a revised SOW as soon as possible.*

There is no indication in EGS that a revised SOW has been submitted and the second annual report submitted by the PI is identical to the first annual report submitted in September 2005."

I agree that the change in research plan represents a profound change, and I described the reason for the change and the changed plan in detail in the first annual report. I am afraid that I did not receive comments on the 2005 annual report in which a revised 'SOW' was requested, and I provide a new SOW here. I hope this SOW will be found acceptable and in a proper format.

Revised SOW

As noted in the 2005 progress report I learned during a visit to Genentech in January of 2003 that scientists and at another lab were pursuing the experiments that had originally proposed. Given their head start and superior resources, I felt that our pursuing the same objective would not be the most efficient use of the Idea award. A strength of my laboratory is expressing difficult proteins for X-ray structural analysis, and we had enjoyed considerable success with structural studies of extracellular regions of HER2/ErbB2 and its homologs. The key remaining questions concerning these receptors deal with exactly how the activity of the kinase domains are regulated. These questions are not simply academic as inhibition of the HER2/ErbB2 kinase by either monoclonal antibodies (Herceptin) or small molecule kinase inhibitors (lapatinib, also known as Tykerb, which was approved last week for treatment of breast cancer) have a proven role in breast cancer therapy, and structural studies of kinases have had a large impact on the design and improvement of kinase-inhibiting drugs. The absence of a crystal structure of the HER2/ErbB2 kinase in any form—native, inhibited, or with substrate bound—despite the intense clinical interest strongly implied that the HER2/ErbB2 kinase is not easy to crystallize. Indeed, previous studies with this kinase indicated poor solubility and difficulty expressing quantities sufficient for structural studies (Jan et al. (2000) Biochemistry 39:9786).

The importance of structural studies of kinases for understanding, designing, and improving kinase-inhibiting drugs has been amply shown by work with the Abl and EGFR/ErbB1 kinases. Structures of kinases in active and inactive conformations and with and without bound drugs have led to deeper understanding of the regulation of kinase activity as well as structure-based design of new inhibitors. More recently, structural studies of drug-resistant kinase mutants that emerge following therapy are also playing an essential role in selecting and designing drugs targeting these resistant kinases. For these reasons, I decided the best use of the Idea Award, both in terms of the intent of the funding mechanism and the strength of my lab, was to initiate structural studies of the HER2/ErbB2 kinase aimed at elucidating the mechanism by which its activity is regulated both naturally and by small-molecule inhibitors. The specific tasks we have pursued to achieve these goals are:

1. Determination of a crystal structure of the human HER2/ErbB2 kinase domain.

1a. Express the human HER2/ErbB2 kinase domain using a baculovirus expression system.
1b. Purify sufficient quantities of the HER2/ErbB2 kinase for structural studies.
1c. Characterize the enzymatic properties and aggregation state of the purified HER2/ErbB2 kinase.
1d. If not monodisperse, identify mutations or solution conditions that result in a soluble, monodisperse form of the kinase.
1e. Crystallize and determine the X-ray structure of the HER2/ErbB2 kinase in native and inhibited states.

2. Determination of the crystal structure of the HER4/ErbB4 kinase region. The EGFR/ErbB1 kinase domain structure has been determined in many states and the HER3/ErbB3 kinase is inactive. By targeting the HER4/ErbB4 kinase as well as the HER2/ErbB2 kinase, we hope to elucidate general and specific features of the three active members of the ErbB receptor family (EGFR/ErbB1, HER2/ErbB2, HER4/ErbB4).
    2a. Express the human HER4/ErbB4 kinase domain using a baculovirus expression system.
    2b. Purify sufficient quantities of the HER4/ErbB4 kinase for structural studies.
    2c. Characterize the biochemical properties and aggregation state of the purified HER4/ErbB4 kinase.
    2d. If not monodisperse, identify mutations or solution conditions that result in a soluble, monodisperse form of the kinase.
    2e. Crystallize and determine the X-ray structure of the HER2/ErbB2 kinase in native and inhibited states.

3. Purification and characterization of intact members of the ErbB family to allow comparison of activities of intact receptor tyrosine kinases with their isolated kinase domains.
    3a. Express the human HER4/ErbB4 kinase domain using a baculovirus expression system.
    3b. Purify sufficient quantities of the HER4/ErbB4 kinase for structural studies.
    3c. Characterize the biochemical properties and aggregation state of the purified HER4/ErbB4 kinase.
    3d. If not monodisperse, identify mutations or solution conditions that result in a soluble, monodisperse form of the kinase.
    3e. Crystallize and determine the X-ray structure of the HER2/ErbB2 kinase in native and inhibited states.

New Result Since September 2006

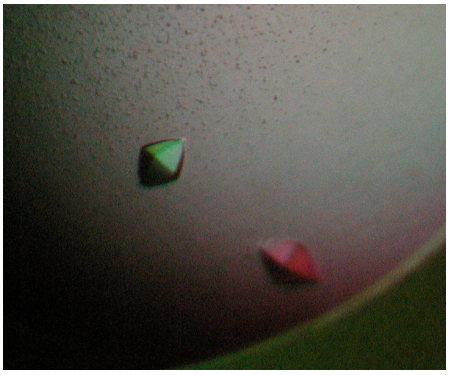

Figure 1. Crystals of the HER4 kinase domain. The longest dimension of the "diamond" is ~0.05 mm.

Since preparation of the 2006 report, crystals of the HER4 kinase region have been grown in the presence of AMP-PNP and $MgCl_2$ (Figure 1). These crystals are small, ~.05 x .04 x .04 mm3, but diffract X-rays to ~3.5 Å using a rotating anode X-ray generator. The space group is primitive hexagonal with unit cell dimensions a= b=103 Å and c=182 Å. We expect that diffraction from the current crystals will extend to higher resolution when more intense synchrotron radiation is used, and the current crystals will suffice for atomic resolution structure determination. Prior to scheduling synchrotron time, however, we are attempting to produce larger crystals by manipulating the crystallization conditions as well as crystal seeding.

Introduction

HER2 (Neu/ErbB2) is a member of the epidermal growth factor receptor (EGFR/ErbB) family of receptor tyrosine kinases (RTKs). RTKs consist of an extracellular ligand-binding region connected to a cytoplasmic tyrosine kinase by a single membrane-spanning region (Burgess et al., 2003). Ligand binding to the extracellular region results in formation of a specific dimeric conformation of the receptor that promotes activation of the cytoplasmic kinase activity. When activated, the kinase phosphorylates specific substrates, including the RTK itself, which initiates a signaling cascade and generally leads to stimulation of cell growth and differentiation. Inappropriate activation of RTKs can lead to uncontrolled cell growth and has been implicated in many human cancers (Tang and Lippman, 1998). Therapeutics targeting EGFR and HER2 have been approved by the FDA for treatment of colon and breast cancer, respectively, and many additional therapeutics targeting EGFR/ErbB family are in the pipeline (Black et al., 2003).

Of the four EGFR homologs found in humans (EGFR/HER1, HER2/Neu, HER3, and HER4), HER2 is unusual in that it lacks a ligand but is the preferred heterodimeric partner of the other EGFR family members. HER2 is of particular clinical importance because it is overexpressed in 20-25% of human breast cancers, and HER2 overexpression correlates with more aggressive tumors and a poorer outcome (Slamon et al., 1987). Therapeutics targeting EGFR/ErbB family members fall into two general categories: monoclonal antibodies directed against the receptor extracellular regions and small-molecule kinase inhibitors. Herceptin (which targets HER2) and Erbitux (which targets EGFR) are monoclonal antibodies currently used to treat breast and colon cancer, respectively. Tarceva and Iressa are small molecule inhibitors of the EGFR kinase that are used to treat lung cancer, and lapatinib (Tykerb) is an inhibitor of both the EGFR and HER2 kinases that was recently shown to delay progression of Herceptin-resistant HER2-positive breast cancers (Authors, 2006). None of these drugs is a cure, however, and resistance develops in each case.

X-ray structural studies of drugs bound to their targets is widely recognized as an important component of rational drug design. We have determined crystal structures of ErbB extracellular regions in the presence and absence of therapeutic monoclonal antibodies, and these results have influenced the design and selection of optimal monoclonal antibodies for ErbB targeted therapies (Burgess et al., 2003). Several crystal structures of the EGFR kinase in the presence and absence of inhibitors have been determined, and these structures have influenced understanding of how the EGFR kinase activity is regulated in both physiological and drug-inhibited states (Stamos et al., 2002; Wood et al., 2004; Zhang et al., 2006). We have thus initiated structural and enzymatic studies of the HER2 and HER4 kinases to better understand the general and

specific features of ErbB kinase activation and inhibition; the HER3 kinase domain is not targeted because it contains four substitutions in its active site believed to be deleterious for kinase activity, and the HER3 kinase has no measurable activity.

We are focusing in particular on crystal structures of the HER2 kinase both alone and complexed with specific inhibitors. These structures will provide insight into the unique features of the HER2 kinase active site, which may be exploited to understand or design HER2-specific inhibitors or modify existing ErbB inhibitors to either narrow or broaden their specificities. Also, resistance to kinase inhibitors commonly occurs through point mutations in the kinase active site (Buschbeck, 2006), and we expect to provide a structural basis for understanding mechanisms of drug resistance and for the design of new generations of inhibitors active against common resistance mutations. Similar structure-based approaches have proven important components of designing second-generation therapeutics effective against Gleevec-resistant forms of the Abl tyrosine kinase, which is the causative agent in chronic myelogenous leukemia (Young et al., 2006).

Body

The aims of the current project are to: **(1)** produce a soluble forms of the HER2 and HER4 kinase domains for structural and enzymatic studies (the EGFR kinase domain will also be produced for comparisons and control experiments), **(2)** perform enzymatic assays of the HER2 and HER4 kinases in the presence and absence of inhibitors and modified inhibitors to vouchsafe the quality of our preparations and aid design and selection of more potent HER2 kinase inhibitors, **(3)** determine the crystal structure of the HER2 kinase domain both alone and complexed with inhibitor compounds that are available both commercially and in the laboratory of our collaborator Phil Cole at Johns Hopkins, and **(4)** produce a full-length form of HER2 for enzymatic and structural studies and comparison with the isolated HER2 kinase domain.

Aim 1. Earlier work on the HER2 kinase domain (HER2KD) has been bedeviled by problems with poor solubility and unstable protein (Jan et al., 2000), and we have also experienced these problems. Solubility and stability did not pose a problem for studies of the EGFR kinase domain, which is well behaved and has been crystallized by three independent groups (Stamos et al., 2002; Wood et al., 2004; Zhang et al., 2006). The EGFR and HER2 kinase domains share 78% amino-acid sequence identity, which, coupled with the known structure of the EGFR kinase domain, allows identification of residues certain to be exposed on the surface of the HER2 kinase. We identified eight solvent-exposed sites that are hydrophobic in HER2 but hydrophilic in EGFR and substituted these sites in HER2 with the EGFR counterpart if basic or Asp if not. Appendix 1 shows an alignment of the amino-acid sequences of the ErbB kinase domains with the residues that were changed in HER2 indicated. We made changes to Asp instead of Glu, Gln, or Asn so as to introduce a charged side chain, which is believed more likely to promote solubility, and avoid "high rotamer" side chains that may present a barrier to formation of stable crystal contacts.

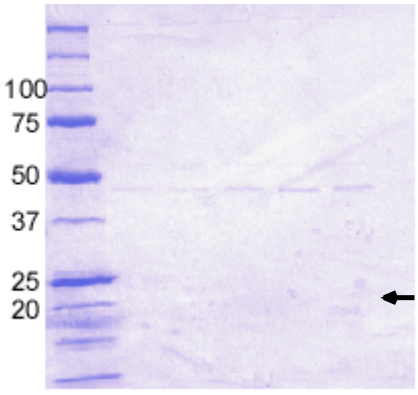

Figure 1. SDS-PAGE analysis of solHER2KD eluted from Streptavidin column. **solHER2KD is indicated by an arrow.**

At the time of last year's report, we had shown by Western blot that we were able to express this mutant "solubilized" form of the HER2 kinase domain (solHER2KD) but had not been able to purify biophysical quantities of the protein. Initial difficulties with purification via a polyhistidine tag have been resolved by incorporation of a streptavidin binding tag (StrepTag), and we are now able to purify ~0.2 mg of solHER2KD per liter of infected Sf9 cells. **Figure 1** shows a Coomassie Blue stained polyacrylamide gel of solHER2KD-containing fractions eluted from a Streptavidin

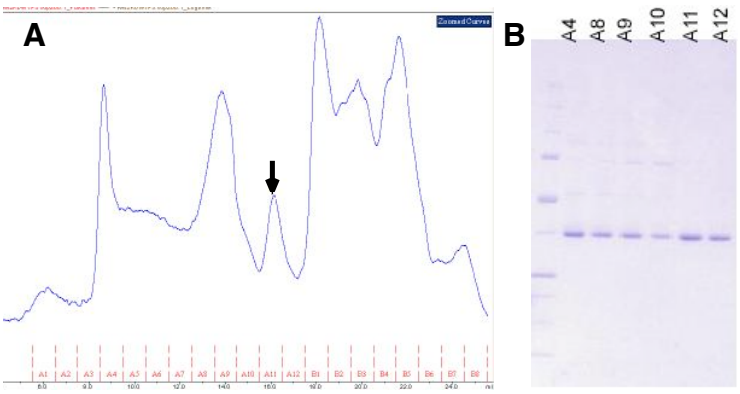

Figure 2. Purification of HER2KD. (A) Size-exclusion chromatography trace of partially purified HER2KD. The monomeric HER2KD peak is indicated by an arrow and comprises fractions A11 and A12. A comparable amount of HER2KD elutes at a position consistent with a HER2KD dimer (fractions A8-A10). (B) SDS-PAGE analysis of fractions from (A). The major band is HER2KD.

column. These fractions have been pooled, concentrated, and subjected to crystallization trials.

As we were working on solHER2KD, we continued working with native HER2KD to enable comparisons of the enzymatic activity of solHER2KD with the wild type kinase. During this work, conditions without added detergent were found in which a substantial fraction of the native HER2KD remained soluble and monomeric as judged by size-exclusion chromatography (**Figure 2**).

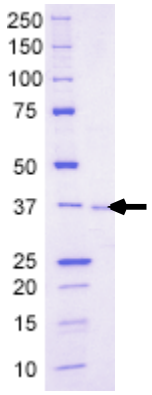

Figure 3. SDS-PAGE analysis of purified HER4KD.

To complete studies of kinase domains of EGFR/ErbB family members and provide a basis for understanding the structural basis for differing substrate and inhibitor specificities among ErbBs, we have also expressed and purified the kinase domain of HER4 (HER4KD) (HER3 lacks kinase activity) (**Figure 3**). The HER4KD is monomeric as judged by size-exclusion chromatography, and the yields of purified HER4KD are comparable to yields of HER2KD and solHER2KD—between 0.2 and 0.3 mg/liter of infected Sf9 cells. Yields are not improved in Hi5 cells.

Aim 2. To verify that our kinase preparations are active, purified HER2KD and HER4KD were subjected to enzymatic assays in which the level of phosphorylation of a biotinylated substrate peptide was measured following incubation with ^{32}P-labeled ATP and purified kinase. K_m and k_{cat} for both ATP and the substrate peptide were determined (**Figure 5**). The values obtained for both kinases are comparable to values previously obtained for related kinases (Hines et al., 2005).

Bisubstrate kinase inhibitors developed in the Cole lab to target the Src and Insulin receptor kinases weakly inhibit this reaction (Hines et al., 2005), but we

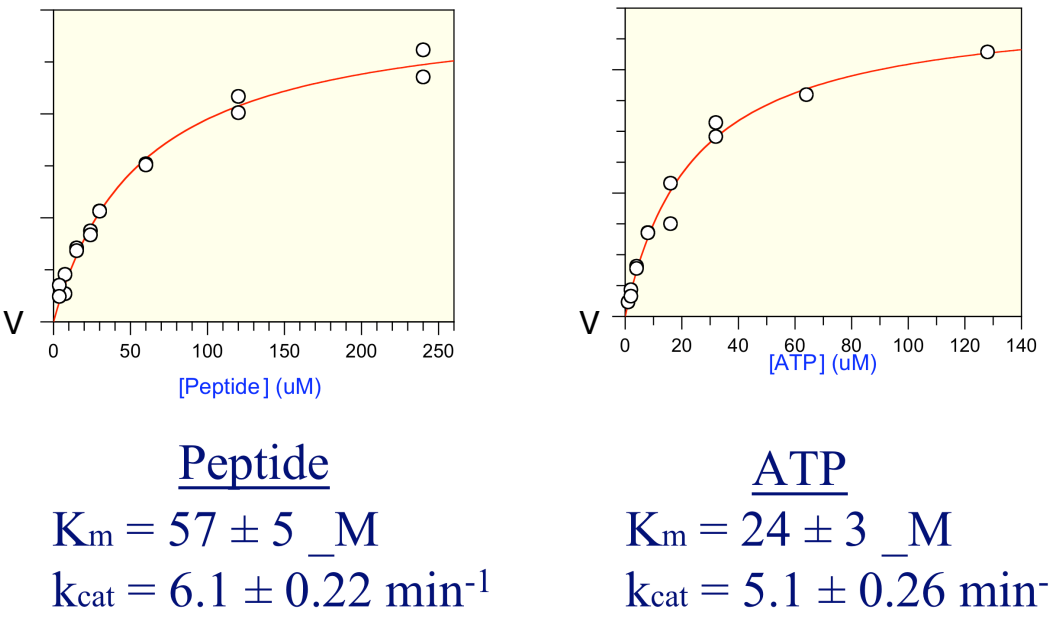

Figure 4. K_m and k_{cat} of HER2KD for ATP and an optimal peptide substrate. Similar results were obtained for HER4KD.

are soon to obtain the HER2 kinase inhibitor lapatinib and will test its ability to inhibit the kinase activity of our preparations shortly. These results show that our purified kinase domains are active and appropriate reagents for both structural and further enzymatic studies.

Aim 3. Crystallization trials with HER2KD, solHER2KD, and HER4KD have been initiated. Small crystals of both HERKD and solHER2KD have been obtained in the presence of ADP (**Figure 5**). Attempts are currently underway to improve these crystals by screening small variations in pH, salt and precipitant concentrations. We recently were informed that an excess supply of the pan-ErbB inhibitor lapatinib will be available to us within a week, and all crystallization trials will be repeated with lapatinib present.

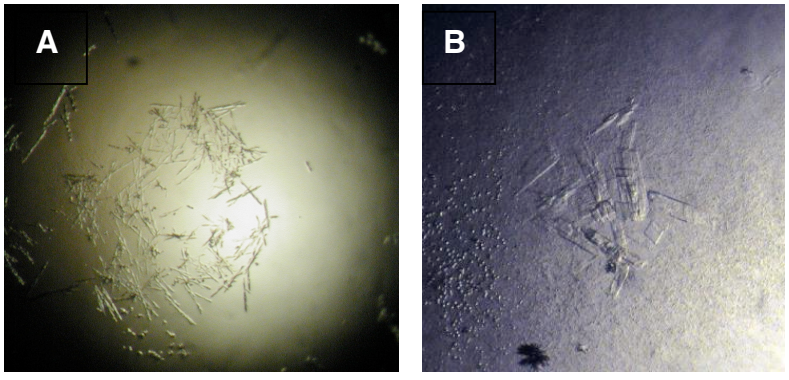

Figure 5. Crystals of (A) HER2KD and (B) solHER2KD.

The current rate-limiting step for our crystallization experiments is production of sufficient protein. To combat this problem we are optimizing viral stock, growth media, and cell culture conditions for large-scale (10 liter) preps. As soon as

larger crystals are obtained, the limit to which they diffract X-rays will be determined using both rotating anode and synchrotron radiation. When crystals that diffract well are obtained, structure determination by molecular replacement should prove straightforward given the large number of homologous structural models in the PDB.

Aim 4. The purpose of this aim is to produce at least microgram quantities of a purified integral membrane form of HER2 so the enzymatic behavior of the isolated ErbB kinase domains can be compared with a more physiological entity. A purified integral membrane form of HER2 will also be used to assess the effects of added regulatory factors—e.g. EGF and EGFR and specific ErbB inhibitors—on the HER2 kinase activity. Our attempts to produce a cell line from which we are able to purify an intact, full-length form of HER2 have been slowed by poor expression levels in transfected cell lines and poor yields during purification. We have been able to express much higher levels of EGFR and have thus focused on developing optimal expression and purification strategies with EGFR, which we then plan to apply to HER2.

Both EGFR and HER2 have C-terminal regions of 200-230 amino acids that harbor several phosphorylation sites. These regions are not believed to adopt a globular structure based on (i) secondary structure predictions, (ii) a paucity of hydrophobic residues, and a much lower lever of sequence conservation among ErbBs within this region relative to other regions of the receptors. This C-terminal region is dispensable for ligand-dependent kinase activity in EGFR and is believed to function primarily as a docking site for downstream effectors of ErbB function. When full-length forms of EGFR and HER2 are expressed, partial proteolysis of this region invariably leads to a heterogeneous receptor population despite multiple strategies to inhibit proteolysis. We have thus expressed truncated forms of EGFR and HER2 that lack this C-terminal region. These receptors are expressed with a polyhistidine tag to aid purification.

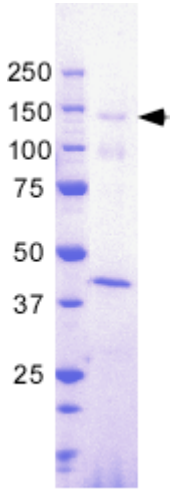

Figure 6. Coomassie Blue stained SDS-PAGE analysis of a partially purified sample of an integral membrane form of EGFR. EGFR is indicated by an arrow, and its identity was verified by Western blot. This EGFR appears to run as a dimer during size-exclusion chromatography.

A cell line overexpressing the truncated EGFR (tEGFR) has been created, and tEGFR has been partially purified by affinity and size-exclusion chromatographies (**Figure 6**). tEGFR appears to run as a dimer on size-exclusion chromatography, consistent with reports that unliganded EGFR may exist as a dimer in cell membranes (Gadella and Jovin, 1995; Moriki et al., 2001). We have been unable to grow our tEGFR expressing cell line

to cell densities greater than 7-8 x 10^5 cells/ml, which limits the amount of tEGFR we can expect to purify, and we are currently investigating expressing tEGFR in HEK293 cells, which we have been able to grow to cell densities >5 x 10^6 cells/ml.

Key Research Accomplishments

1. Purification of near milligram amounts of HER2KD, solHER2KD, and HER4KD

2. Growth of preliminary crystals of HER2KD and solHERKD.

3. Demonstration of normal kinase activity levels for purified HER2KD and HER4KD, and inhibition of this activity by bisubstrate inhibitors developed in the Cole lab.

4. Production of the Y877F HER2KD mutant to study the effects of phosphorylation at Tyr 877 on HER2KD activity.

5. Partial purification of tens of micrograms of intact EGFR.

Reportable Outcomes

None so far.

Conclusions

1. Sufficient amounts of HER2KD, solHER2KD, and HER4KD for structural and biophysical studies can be purified.

2. Purified HER2KD and HER4KD (and probably solHER2KD) are enzymatically active.

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Appendix

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HER2 SOL Subs     D                                        K       D   D
HER2_human      GAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPVAIKVLRENTSPKA 60
EGFR_human      GEAPNQALLRILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREATSPKA 60
HER4_human      GTAPNQAQLRILKETELKRVKVLGSGAFGTVYKGIWVPEGETVKIPVAIKILNETTGPKA 60
HER3_human      -EKANKVLARIFKETELRKLKVLGSGVFGTVHKGVWIPEGESIKIPVCIKVIEDKSGRQS 59
                   .*:.  **:****:::******.****:**:*:*:**.:****.** :.: :. ::

HER2 SOL Subs               D
HER2_human      NKEILDEAYVMAGVGSPYVSRLLGICLTSTVQLVTQLMPYGCLLDHVRENRGRLGSQDLL 120
EGFR_human      NKEILDEAYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLDYVREHKDNIGSQYLL 120
HER4_human      NVEFMDEALIMASMDHPHLVRLLGVCLSPTIQLVTQLMPHGCLLEYVHEHKDNIGSQLLL 120
HER3_human      FQAVTDHMLAIGSLDHAHIVRLLGLCPGSSLQLVTQYLPLGSLLDHVRQHRGALGPQLLL 119
                   . *.   :..:. .:: ****:*  .::**:** :* *.**::*::::. :*.* **

HER2 SOL Subs                R
HER2_human      NWCMQIAKGMSYLEDVRLVHRDLAARNVLVKSPNHVKITDFGLARLLDIDETEYHADGGK 180
EGFR_human      NWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTPQHVKITDFGLAKLLGAEEKEYHAEGGK 180
HER4_human      NWCVQIAKGMMYLEERRLVHRDLAARNVLVKSPNHVKITDFGLARLLEGDEKEYNADGGK 180
HER3_human      NWGVQIAKGMYYLEEHGMVHRNLAARNVLLKSPSQVQVADFGVADLLPPDDKQLLYSEAK 179
                ** :****** ***:  :***:********:*:*.:*::::***:* **  ::.:   . .*

HER2 SOL Subs                                    D
HER2_human      VPIKWMALESILRRRFTHQSDVWSYGVTVWELMTFGAKPYDGIPAREIPDLLEKGERLPQ 240
EGFR_human      VPIKWMALESILHRIYTHQSDVWSYGVTVWELMTFGSKPYDGIPASEISSILEKGERLPQ 240
HER4_human      MPIKWMALECIHYRKFTHQSDVWSYGVTIWELMTFGGKPYDGIPTREIPDLLEKGERLPQ 240
HER3_human      TPIKWMALESIHFGKYTHQSDVWSYGVTVWELMTFGAEPYAGLRLAEVPDLLEKGERLAQ 239
                 ********.*    :************:*******.:** *:   *:..:*******.*

HER2 SOL Subs                               D
HER2_human      PPICTIDVYMIMVKCWMIDSECRPRFRELVSEFSRMARDPQRFVVIQ-NEDLGPAS-PLD 298
EGFR_human      PPICTIDVYMIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQGDERMHLPS-PTD 299
HER4_human      PPICTIDVYMVMVKCWMIDADSRPKFKELAAEFSRMARDPQRYLVIQGDDRMKLPS-PND 299
HER3_human      PQICTIDVYMVMVKCWMIDENIRPTFKELANEFTRMARDPPRYLVIKRESGPGIAPGPEP 299
                * ********:******** : ** *:**  **::***** *::**: :.    .. *

HER2 SOL Subs              D             * End of Kinase Domain
HER2_human      STFYRSLLEDDDMGDLVDAEEYLVPQQG------FFCPDPAPGAGGMVHHRHRSSSTRSG 352
EGFR_human      SNFYRALMDEEDMDDVVDADEYLIPQQG------FFS----------------SPSTS-- 335
HER4_human      SKFFQNLLDEEDLEDMMDAEEYLVPQAFNIPPPIYTSRARIDSNRSEIGHSPPPAYTPMS 359
HER3_human      HGLTNKKLEEVELEPELDLDLDLEAEEDNLATTTLGSALSLPVGTLNRPRGSQSLLSPSS 359
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